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LEAF DIFFERENTIATION IN ANGIOSPERMS*

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INTRODUCTION

The foliage leaf of angiosperms is so widely used in modern anatomical, physiological and genetical research that definite information regarding its growth and differentiation is highly desirable. An examination of modern botanical texts, however, reveals a surprising neglect of even the more general aspects of this problem although tissue differentiation in the stem and root is usually treated in considerable detail. This circumstance has prompted the present review which attempts to summarize and to correlate the most important of the recent studies on foliar histogenesis.

The task of integrating the widely scattered literature is difficult, particularly because of the disproportionate emphasis which has been placed recently upon the mode of origin of the leaf at the growing point. As a consequence, the later significant phases in foliar differentiation are often minimized or completely neglected, so that comparatively little information is available on the *entire* career of development of the various types of angiospermous leaves. Under such circumstances, the reviewer disagrees with the opinion recently expressed by Troll (65) that the "ontogenetic method" is subordinate in importance to the comparative or "typological method" in the study of the leaf. On the contrary, it would seem obvious that a sound and well balanced morphological treatment must attempt to relate the facts of development *impartially* to the form and structure of the adult organ. Schüepf's (55) ideas in this connection are pertinent and timely. He states: "Our comparative morphology deals always with adult forms without proper regard to their method of origin. However, concepts which

*I am grateful to my wife for her valuable assistance in preparing the illustrations and diagrams in this article. Thanks are also due to Professor D. R. Hoagland and H. S. Reed for reading the manuscript.

describe form should be replaced by concepts which deal with the regulation of growth."

In the following pages, the literature will be discussed according to the successive phases through which a leaf passes from inception to maturity. This method of treatment has two important advantages: (1) it serves to emphasize the essential relationship between the successive stages in leaf development, and (2) it permits, under each differentiation phase, of a brief indication of the problems requiring further investigation.

LEAF INITIATION AT THE GROWING POINT

The classical investigations of Hanstein (22) definitely established the fact that the angiosperm leaf originates from the outer layers of cells at the side of the shoot growing point. This principle of development has many significant implications. From a physiological standpoint it is clear that the factors determining the position, sequence and character of foliar primordia at the growing point are, in the final analysis, those which regulate the growth and division of cells in primordial meristematic tissue. In several recent papers Priestley (43, 44) has analyzed certain of the possible factors which may govern the shape, plane of division, and mode of differentiation of cells in the shoot apex and Zirkle (70) has given us valuable data on the cytology of primordial meristem and its derivatives. It is obvious, however, that at present we are merely on the fringe of a complex and involved series of problems, the solution and integration of which depend upon further knowledge of protoplasmic behavior.

From an anatomical standpoint, Schüepp (52) has advanced a useful theory to account for the emergence of foliar primordia at the shoot apex. He points out that since the intensity of cell division is approximately the same in all portions of the growing point, adjustment to the continued increase in volume is brought about by folding of the outer cell layers at definite points. These surface folds represent leaf primordia which thus appear as the inevitable outcome of the periclinal growth characteristic of primordial meristem. Schüepp's viewpoint has been adopted by Priestley (43, 44, 45) and Pottier (41) and in the reviewer's opinion deserves serious consideration in any effort to visualize the forces underlying the growth and behavior of primordial meristem in the shoot apex.

It must be realized, however, that the typical "surface folding" just described is usually accompanied by distinctive periclinal divisions in certain of the outer cell layers. Hanstein's (22) observations showed that such divisions are confined to what he termed the "periblem" or subepidermal layers of the growing point. Subsequent investigations have indicated that in some cases, at least, the internal cells of the leaf primordium originate exclusively from periclinal divisions in the outermost periblem layer which are accompanied by the anticlinal division and surface growth of the dermatogen or young epidermis. This condition occurs in *Elodea*, *Galium* and *Hippuris* (24) and in certain marine genera of the family *Potamogetonaceae* (41). In *Ceratophyllum* (26), however, the "plerome" of the leaf "arises from an outer cell of a strand of plerome cells radiating from the stem plerome to the leaf primordium."

Within recent years, however, interest in the problem of leaf initiation and differentiation in other types of angiosperms has been stimulated through a study of certain periclinal chimaeras.¹ Stated briefly, the growing point of a typical periclinal chimaera is composed of one or more external layers of cells, derived from one parent, and a central mass contributed by the other graft component. Thus, depending upon the number of external layers belonging to a given parent, one layered (haplochlamydous), two layered (diplochlamydous), and even three layered (triplochlamydous) periclinal chimaeras are recognized by Winkler (67) in *Solanum*. In a haplochlamydous chimaera, such as *S. tubingense* and *S. Koelreuterianum*, the form of the leaf is only slightly influenced by the outer single-layered component since the bulk of the primordium is derived from the inner subepidermal layers of the growing point (32). In a diplochlamydous chimaera, however, it is obvious that the periclinal divisions associated with leaf initiation must occur *at first* in the third layer of the growing point, resulting in the elevation and incorporation of the two external layers in the primordium. Such is the case with the diplochlamydous *Solanum* chimaeras (28, 32) and with the *Crataegomespili* (33). The work of Krumbholz (29), likewise, indicates the possibility of such chimaeras in *Oenothera*.

¹ For a summary of the genetical and anatomical aspects of periclinal chimaeras, cf. Jones (27) and Winkler (67).

These studies, as well as others conducted under the stimulus of the theory of periclinal chimaeras, demonstrate a wide variation in respect to the rôle of the various meristematic layers in leaf and bud initiation. For example, Noack (39) concluded that in *Pelargonium zonale* "the whole mass of the leaf, including the petiole, arises from a single subepidermal layer of the shoot apex." A similar conclusion was reached by Schwarz (57) in his investigation of leaf development in *Plectranthus fruticosus* and *Ligustrum vulgare* and by Halmai (21) in a study of *Centaureum*. On the other hand, the investigations of Massey (37) on *Arabis albida* and *Euonymus japonicus*, those of Kühn (30) on *Nepenthes* and Weidt's (66) study on *Heterotrichum macrodon* Planch., clearly show that the third layer of cells at the growing point is concerned in leaf formation. A somewhat intermediate condition occurs in *Hypericum uralum*, according to Zimmerman's (69) detailed observations, in that derivatives of the third layer form only the lower portion of the midrib while the remaining internal leaf tissue arises from the subepidermal layer.² Less attention seems to have been devoted to the situation in monocotyledons but the available data likewise indicate considerable variation in this group. Rösler (48), for example, found that the leaf of *Triticum vulgare* arises exclusively from the outermost cell layer or dermatogen, while Priestley, Scott and Gillett (46) state that in *Alstroemeria aurantiaca* Don., "the first appearance of a new primordium is a slight upfold of the superficial tissues, due in part to periclinal divisions in the dermatogen layer as well as in the underlying layers."

On the basis of the above studies, it is obviously impossible to generalize as to the most common scheme of leaf initiation in the angiosperms (6). On the contrary, it seems clear from Schmidt's (50) careful investigations that the mode of leaf formation is directly related to the architecture of the growing point in each particular case. According to Schmidt, the shoot apex consists of one or more self-perpetuating peripheral layers of cells which he collectively terms the *tunica*; periclinal divisions normally appear in certain of these layers only during leaf or axillary bud formation. The inner core of the growing point, in which growth in volume predominates, he designates as the *corpus*.³ Schmidt em-

² A similar condition prevails in *H. acutum* and *H. montanum*, according to Herbst (23).

³ Schmidt's terminology has been widely adopted (12, 20, 21, 30, 34, 48, 69) and in the reviewer's opinion deserves general acceptance by anatomists.

phasizes that the extent to which tunica and corpus participate in leaf or bud initiation depends upon the quantitative relationships of these regions in a given shoot apex. Thus, in *Scrophularia nodosa* L. the tunica is represented by a single layer corresponding to the dermatogen and the leaf arises from the outer portion of the corpus. In contrast, the growing point of *Vinca minor* L. possesses a three layered tunica from the inner layers of which the leaf originates. An intermediate condition is illustrated by *Veronica myrtifolia* Sm. where both tunica and corpus derivatives form the leaf initial. This latter condition is similar to Foster's (12) description of bud scale and foliage leaf emergence in *Carya* (fig. 1) and to the origin of the leaf in *Oenothera* (29).

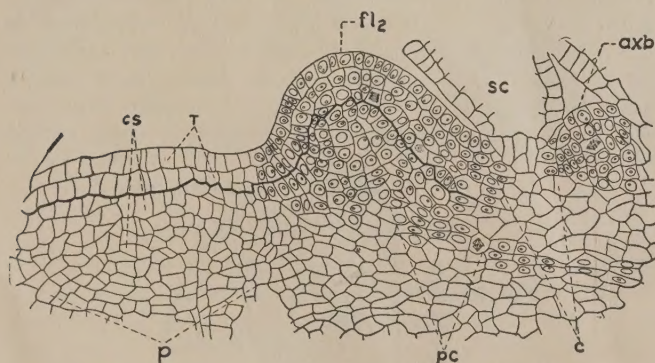


FIG. 1. (From Foster (12)). Median longitudinal section through the growing point of *Carya Buckleyi* var. *arkansana*, showing an early developmental stage of a foliage leaf. Note that derivatives of both the tunica (*t*) and corpus (*cs*) are present in the primordium. Legend: *axb*, primordium of staminate catkin bud; *c*, cortex of internode; *fl*, foliage leaf primordium; *p*, pith of stem; *pc*, procambial tissue; *sc*, portion of bud scale. ($\times 900$).

ORIGIN AND EARLY DIFFERENTIATION OF PROCAMBIUM

For a short time after its emergence from the growing point, a foliar primordium consists of actively dividing primordial meristem cells. One of the first significant indications of specific differentiation following this phase consists in the formation of the median procambial strand near its base (fig. 1). According to Yarbrough (68), this occurs in *Bryophyllum calycinum* when the primordium "is between 60 and 70 μ long." In *Alstroemeria*,

Priestley, Scott and Gillett (46) found both median and lateral procambial strands in a primordium only $54\ \mu$ long.

The general importance of this early appearance of procambial tissue has been emphasized by several investigators. Priestley and Scott (45) maintain that procambial cells "probably exercise a very great influence on further development and their presence is probably responsible for the fact that the leaf primordium now grows much more rapidly than the meristematic apex." The most comprehensive study of the morphogenesis of procambial tissue in the shoot apex, however, has recently been made by Louis (34) who has described and profusely illustrated the condition in various types of angiosperms as well as in *Taxus*, a gymnosperm. Confirming the earlier ideas of Gregoire (18, 19), Louis finds that the actual emergence of each foliar primordium is preceded by the transverse expansion of the shoot apex to form two (decussate-leaved plants) or one (alternate-leaved plants) axial portions which he designates as "foliar buttresses" ("soubassements foliaires"). Each foliar buttress with its vertically emergent primor-

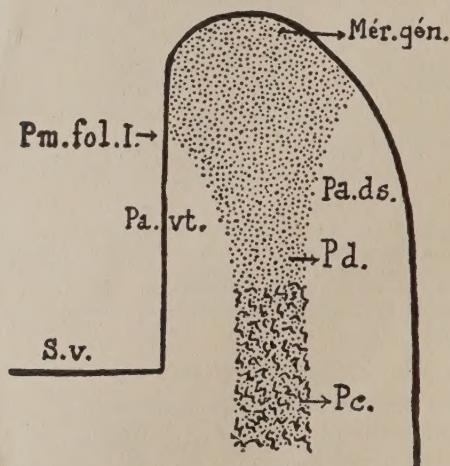


FIG. 2. (From Louis (34)). Schematic reconstruction of a median longitudinal section of a leaf primordium of *Syringa vulgaris*, showing the differentiation of procambium from prodesmogen. Legend: *mér. gén.*, general meristem; *Pa. ds.*, dorsal parenchyma; *Pa. vt.*, ventral parenchyma; *Pc.*, procambium; *Pd.*, prodesmogen; *Pm. fol I.*, leaf primordium; *S. V.*, growing point.

dium is thus regarded as a unit, an idea quite comparable to Priestley's (44, 46) "growth units" which consist of a leaf initial and a segment of the shoot axis (*cf.* also Schüeppe (56)). According to Louis, the early dorsal and ventral vacuolation of cells in the lower portion of an elongating primordium results in the demarcation of a central "arc" of meristem which he terms the "prodesmogen." As figure 2 shows, the first true procambium subsequently originates from this prodesmogen. Since the further differentiation of procambium proceeds both acropetally into the primordium and basipetally into the foliar buttress, vertical "leaf traces" are produced. Thus, the earliest formed procambium in the stem consists of discrete strands (separated by undifferentiated prodesmogen) which arise in connection with the foliar buttresses and their emergent primordia. The number of strands present in each case is directly related to the phyllotaxis and the relative width of insertion of the primordia. In the reviewer's opinion, the data and ideas presented by Louis provide a basis for a truly objective study of shoot organization in the angiosperms. It seems clear that any effort to isolate the leaf or stem, at least from a morphogenetic standpoint, is unsupported by the facts of development.

ORIGIN OF PETIOLE, MIDRIB, AND LAMINA

Soon after its appearance at the growing point, the pad-like or peg-shaped leaf primordium embarks upon a definite career of morphological differentiation, which consists in the initiation of such parts as the petiole, lamina, sheath, stipules, etc. Thus, the basic architecture of the adult leaf originates very early in ontogeny, a fact of considerable significance to the general problem of form determination (11, 12). Early studies of leaf development, such as those of Trécul (62), Eichler (8), Goebel (15), Deinega (7) and Massart (36), were concerned largely with the *external aspects* of morphological specialization and neglected the accompanying process of internal cellular differentiation in the primordium. Unfortunately, a similar criticism can be applied to much of the modern work on leaf development. Troll (63, 64), for example, has very recently discussed in great detail the comparative morphology and form development of the peltate and pinnate type of leaf although he entirely ignores the histogenetic implications of his data. As a result of the dominance of this one-sided atti-

tude, our knowledge of the cellular differentiation of the various morphological regions of a leaf is limited to a small number of recent investigations, the results of which will now be summarized.

The available data clearly show that in many dicotyledons the formation of the lamina is preceded by the differentiation of the future petiolar-midrib region. The latter arises as the result of the early growth in length of the primordium which often acquires at this stage the form of a tapering, adaxially-flattened cone. Certain phases in the differentiation of the petiolar-midrib region have been studied by several investigators, particularly with reference to the processes of apical growth. In *Pelargonium* (39) and in *Plectranthus* and *Ligustrum* (57) apical growth is related to the continued periclinal and anticlinal division of subepidermal initials; the latter are lineal descendants of the subepidermal cell-layer of the growing point. A similar condition, according to Avery (2), occurs in the tobacco leaf where "the addition of cells at its apex may be traced to the activity of a single subepidermal cell." Other investigators, however, maintain that apical growth is not necessarily limited to the division of subepidermal initials at the leaf apex. Krumbholz (29) found that in *Oenothera* active cell divisions occur both apically and subapically, a view supported by Lange's (32, 33) detailed analysis of leaf development in *Solanum chimaeras* and in the *Crataegomespili*. It will be recalled that in the examples just mentioned, more than two layers of cells are involved in the initiation of the leaf. In all such cases, as Lange (32) emphasizes, the degree to which the derivatives of the various subepidermal layers of the shoot apex participate in apical growth varies considerably and may be traced with confidence only in certain diploclamydous chimaeras. Similarly, Foster (12) has shown that at an early stage in the differentiation of bud scale and foliage leaf in *Carya*, no clear demarcation can be made between the derivatives of the inner layer of the tunica and the corpus in the apical region of the primordia. We may, therefore, conclude that the process of cell division in the leaf apex of angiosperms follows no universal scheme. On the contrary, true apical growth ceases relatively early and the subsequent intercalary extension of the petiolar-midrib region is accompanied by general cell division and cell extension, regardless of the histogenetic mode of origin of the leaf (2, 12, 25, 60, 66, 68).

The apical and intercalary extension of the petiolar-midrib region is accompanied in many cases by a characteristic increase in radial thickness. This type of growth is associated particularly with the activity of a vertical strip of periclinally dividing cells found beneath the adaxial epidermis. As a result of a broad comparative study, Bouygues (4) found that this type of cambial-like thickening is typical of the early phases of petiole development in many types of dicotyledonous leaves. Schüepp (53), likewise, has pointed out that in *Acer pseudoplatanus* L. the petiole arises, not as the primary result of the intercalary elongation of a zone interpolated between lamina and leaf base,⁴ but because of a growth in thickness "involving almost the entire inner surface of the leaf base." McCoy (35) also states that the petiole of the leaf of *Zeugites* "thickens by an extended period of secondary cell division in its ground tissue," a situation which is duplicated in the leaves of certain aroids (7). In various species of *Carya* (12, 13) the early marked increase in radial thickness due to this adaxial strip of meristem produces a massive leaf-axis or phyllopodium (5) which later specializes into the leaf-base, petiole and rachis of the pinnate leaf; a similar type of meristematic activity also occurs in the midrib of each leaflet (fig. 3).

Following the early specialization of the petiolar-midrib region of most simple leaves, the lamina begins to differentiate from its upper portion as two thin marginal ridges of meristem (fig. 3). The stage at which laminar differentiation begins varies considerably. Marginal growth in *Oenothera* (29) and in *Plectranthus* (57) occurs when the primordium is less than .1 mm. in height, and in *Bryophyllum* it "is evident as early as the 350 μ stage" (68). In *Nicotiana* (2), however, the lamina does not appear until the embryonic midrib is approximately .6 mm. long. As in the case of apical growth, divergent ideas exist as to the importance of the subepidermal layer of the midrib in the origin and marginal growth of the young lamina. Gidon (14), Noack (39), Schwarz (57), Avery (2), Johnson (25) and Weidt (66) maintain that the differentiation of the lamina may be traced to the activity of a band of submarginal cells at the edge of the midrib. A similar condition appears to explain the origin and early growth of the lamina from

⁴ This interpretation of petiole development originated with Eichler (8) and dominates Goebel's (16) viewpoint of foliar ontogeny.

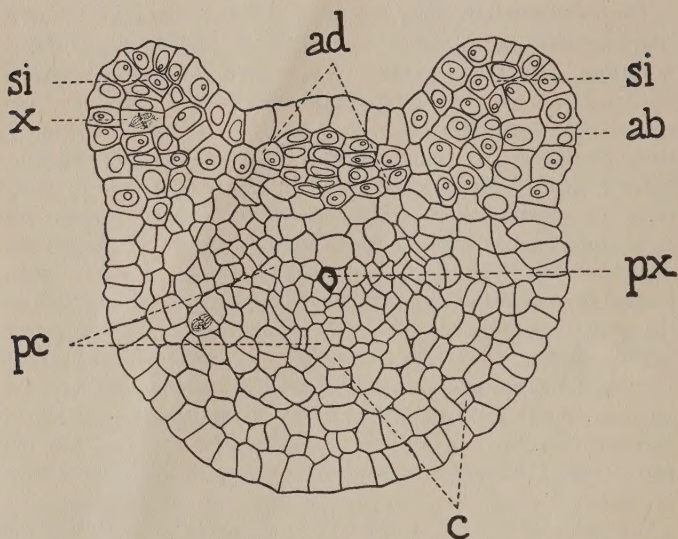


FIG. 3. Transverse section near base of median leaflet (224μ high) of a young seedling foliage leaf of *Carya Buckleyi* var. *arkansana*, showing the origin of the lamina from the adaxial margins of the midrib. Note that each lamina half consists of three internal layers of meristematic cells enveloped by the epidermis. The periclinal division in cell X illustrates the point of origin of the middle layer. Legend: *ad*, adaxial meristem of midrib; *ab*, abaxial surface of lamina half; *c*, cortex of midrib; *pc*, procambium; *px*, protoxylem element; *si*, submarginal initial of lamina. ($\times 900$).

the leaflet-midribs in various species of *Carya* (12, 13, 31). Krumbholz (29) and Lange (32), while admitting the importance of such localized marginal growth, contend that both subepidermal, as well as deeper, cell layers may participate to varying degrees in lamina formation (*cf.* also Kühl (30)). Additional studies on the complete developmental history of the leaf in a wide range of angiosperms are obviously needed before any general conclusions can be reached. As an indication of the necessity for more data may be cited the recent observations of Renner (47) on lamina differentiation in a white-margined haplochlamydous chimaera of *Sambucus nigra*. According to Renner, the edge of the young lamina is provided with a strip of periclinally-dividing dermatogen cells, which contribute to the colorless marginal region of the leaf. Although periclinal divisions in the marginal dermatogen cells occur during

lamina differentiation in certain monocotyledons (41), Renner is one of the first to describe a similar situation for a dicotyledon. A thorough study of both normal and periclinally-variegated leaves in dicotyledons would possibly bring to light additional examples of this condition.

The differentiation of petiole, midrib and lamina described above appears characteristic of many simple leaves and corresponds to what Prantl (42) termed the "pleuroplastic type" of development. Almost no information exists, however, regarding the cellular differentiation of pinnate, digitate (*i.e.*, "palmate") or pedate leaf types, in spite of their widespread occurrence in dicotyledons. According to Troll's (64) recent monograph, the leaflets (or lobes) of such leaves may arise in three possible ways; *viz.*: (a) in *basipetal sequence*; *i.e.*, from the apex towards the base, (b) in *acropetal sequence*; *i.e.*, from the base towards the apex, and (c) in *divergent sequence*; *i.e.*, the first pair of leaflet primordia arises near the middle region from which point additional pinnae develop both acropetally and basipetally. The "basipetal leaf" seems to dominate in the angiosperms and represents, in Troll's (64) opinion, the "basic type" to which may be traced the manifold character of pinnate leaves (*cf.* also Troll (65)). The differentiation of the typical acropetal leaf of *Carya* has been described by Foster (12, 13) who finds that the lateral leaflet initials arise as hemispherical protuberances from the primordial meristem at the margins of the leaf axis, the apex of which produces the terminal leaflet (*fig.* 4). As far as the reviewer is aware, however, no similar study has been made of either a basipetal or divergent type of pinnate leaf. The problem clearly requires further histogenetic investigation, especially since Troll states that the basipetal and acropetal order of leaflet formation is usually correlated respectively with an early or late cessation of apical growth in the primordium as a whole. With this idea as a basis, it should be possible to connect the histogenetic process with the elaboration of form in the lamina.

HISTOGENESIS AND GROWTH OF THE LAMINA

Recent investigations on the origin and differentiation of tissues in the lamina have raised many points of considerable anatomical, physiological and genetical interest.⁵ The most important results

⁵ The older, scattered literature in this field has been discussed by Avery (2) and Smith (59).



FIG. 4. (From Foster (12)). Longitudinal section through a foliage leaf (288 μ high) of *Carya Buckleyi* var. *arkansana*, illustrating the acropetal formation of the lateral leaflet primordia. Legend: *c*, cortex of leaf axis; *h*, hair; *ll*₁-*ll*₃, primordia of lateral leaflets; *tl*, terminal leaflet. ($\times 900$).

of these studies may be conveniently summarized under three main topics:

1. *Marginal growth.* Smith (59) has shown in a recent comparative survey that the embryonic lamina consists of 5–8 distinct layers of meristematic cells which ultimately differentiate into the epidermis, mesophyll and vascular bundles of the adult leaf blade. The origin and behavior of the typical stratified meristem of the young lamina and its relation to the adult "tissue pattern" have been studied by other investigators in a number of species and the data are presented schematically in figures 6 and 10. In all these cases, a distinctive type of "marginal meristem" is present, from which the various embryonic layers originate. The outermost cells of this meristem represent the "marginal initials" (Mi in all figures) and give rise to the upper and lower dermatogen layers which perpetuate themselves by anticlinal divisions and surface growth. Similarly, the continued formation of the internal layers may be traced to the activity of a band of "submarginal initials." (Si in all figures). The diagrams, however, clearly indicate two distinct types of behavior of the submarginal initials. In one type, as illustrated by *Bougainvillea* (14), *Pelargonium* (39), *Plectranthus* and *Ligustrum* (57), *Nicotiana* (2), and *Kalanchoë* (25), the sub-



FIG. 5. (From Avery (2)). Transverse section of outer edge of young lamina of *Nicotiana tabacum*, showing anticlinal division of submarginal cell into cells A and B, and the genesis of the internal layers. Legend: *u ep*, upper epidermis; *u m*, upper or adaxial mesophyll layer; *m m*, middle mesophyll layers with young vein; *l m*, lower or abaxial mesophyll layer; *l ep*, lower epidermis.

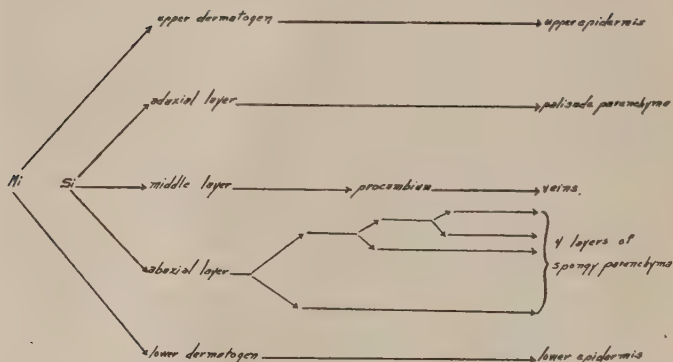


FIG. 6. Schematic representation of tissue differentiation in the lamina of *Bougainvillea spectabilis*. (Based on Gidon (14)).

marginal initial divides at right angles to the surface of the blade, forming an outer and an inner cell (cells A and B of fig. 5). From the inner cell (B) the middle layer or layers of the lamina are derived. The outer cell (A) meanwhile divides parallel to the leaf surface, one daughter cell persisting as an initial, the other being added either to the abaxial or adaxial layer. Thus in this type, anticlinal and periclinal division-planes alternate in the sub-marginal initial and the origin of the main internal layers may be traced to the very edge of the lamina (fig. 5). According to Avery

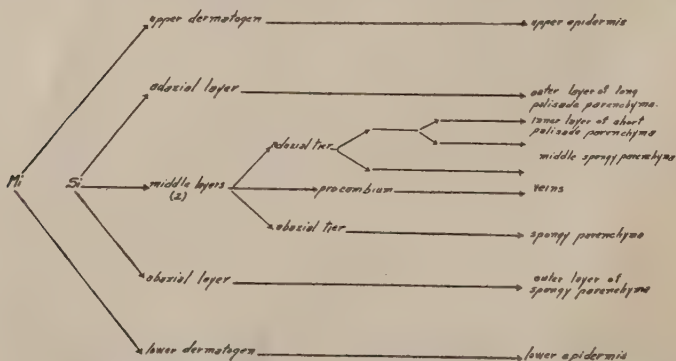


FIG. 7. Schematic representation of tissue differentiation in the lamina of *Pelargonium zonale*. (Based on Noack (39)).

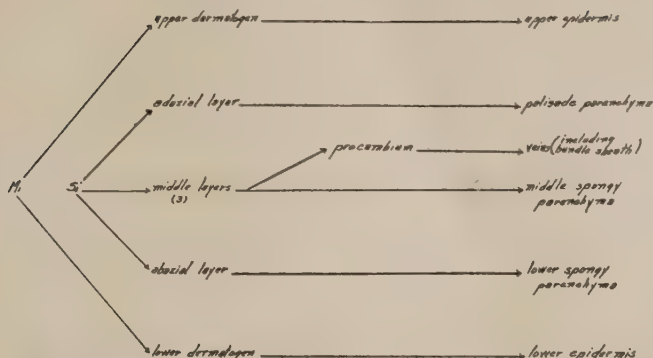


FIG. 8. Schematic representation of tissue differentiation in the lamina of *Nicotiana tobacum*. (Based on Avery (2)).

(2), this characteristic behavior of the submarginal initials in *Nicotiana* "apparently continues as long as there is marginal growth." A contrasted type of submarginal activity is illustrated by *Carya* (12, 13) and by two species of the Melastomaceae; viz., *Heterotrichum macrodon* Planch and *Clidemia hirta* Don. (66). Here the submarginal initials, by alternating oblique divisions, first produce two internal layers of cells (figs. 9-10). At a varying distance from the leaf margin, the abaxial layer divides periclinally, thus producing an inner or middle tier of cells (cf. fig. 3).

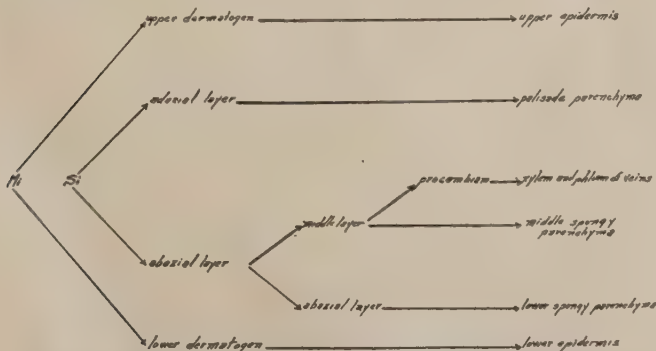


FIG. 9. Schematic representation of tissue differentiation in the lamina of *Carya Buckleyi* var. *arkansana*. (Based on Foster (12)).

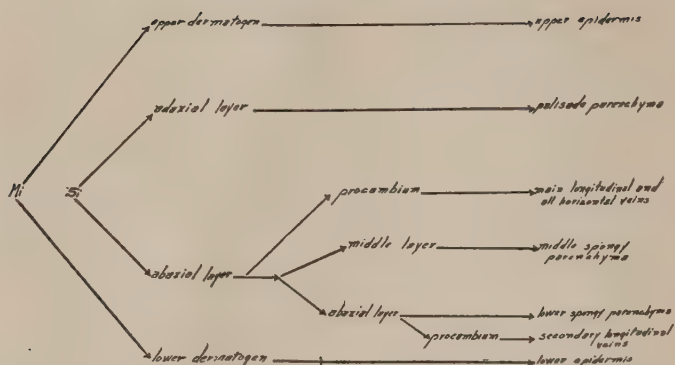


FIG. 10. Schematic representation of tissue differentiation in the lamina of *Heterotrichum macrodon*. (Based on Weidt (66)).

The distribution of these two distinct types of marginal growth in the angiosperms deserves careful investigation. That other modes of lamina formation exist is indicated by Skutch's (58) observations on the leaf of *Musa sapientum* L. Here the initiation of the lamina-halves occurs at a relatively late stage "when the marginal cells of the midrib have lost their meristematic nature and have differentiated into a scarious border." Thus in *Musa*, lamina differentiation is relegated to deeper layers and a strictly marginal meristem is absent.

2. *Surface growth and the regulation of form.* True marginal growth in the lamina is of short duration as compared with the protracted surface growth characteristic of later phases in development. This is clearly shown in the "basipetal type" of leaf where the cessation of marginal activity proceeds from the apex towards the base. Thus in *Pelargonium*, marginal growth ceases when the main lobes of the lamina have been formed (39). A similar early maturation of the marginal meristem likewise obtains in other basipetal types (66), so that all further growth in the area of the lamina is due largely to cell division and cell extension throughout the embryonic layers, to which Schüepp (52, 54) has given the collective term of "plate meristem." Cell division in the various layers of the plate meristem is predominantly anticlinal in respect to the surface of the lamina, with the result that the characteristic number of layers established by marginal growth is relatively constant

throughout the development of the leaf (2, 12, 51, 66). The only important disturbance in the typical stratified appearance of the plate meristem during surface growth results from the early differentiation of procambial strands in certain of the layers (fig. 5). However, the areas between procambial strands or even well-differentiated vascular bundles remain undifferentiated and capable of prolonged growth (12). As Schüepp (51) has shown in *Acer pseudoplatanus* L., the characteristic plicate veneration of the lamina in the bud is related to the adjustment between surface growth and the longitudinal extension of the prominent ribs of the main veins.

It is extremely difficult to visualize the complex relation of marginal and surface growth to the adult form of the lamina. Tetley (61) suggests that "the balance between the rate of differentiation and the capacity for extension of the marginal cells on the one hand, and the rate of differentiation of the mesophyll within is evidently an important factor in determining the size and shape of the leaf." Avery (2) has approached the problem of leaf form in *Nicotiana* from the standpoint of the relation of differential rates of growth in various parts (or "segments") of a developing lamina to the growth of the leaf as a whole. The ratio between the relative growth of any measured segment of the leaf and the total area of the blade during various stages in development, is expressed mathematically by the symbol " k ." Avery has determined the values for k throughout the growth of the leaf and concludes from his data that "localized growth" (*i.e.*, differential distribution of growth in various portions) and "polarized growth" (*i.e.*, greater growth in one dimension than in another) are responsible for the final shape of the organ. More recently, Avery (3) has investigated the role of auxin a in the growth and differentiation of the tobacco leaf. His data suggest that auxin may be partly responsible for polarized growth although the effect of this hormone on localized growth remains to be investigated. Avery's work clearly indicates the need for a careful examination of the implications of modern hormone research on developmental problems. In addition to hormones, the relation of certain microelements, such as copper, zinc and manganese, to leaf growth deserves investigation. Obviously, in view of the great variation in the distribution of growth in the lamina of dicotyledons (40, 42), the whole subject challenges anatomical and physiological technique.

3. *Differentiation and maturation of tissues.* The destiny or "prospective significance" of the internal layers⁶ of the young lamina represents a problem of considerable morphogenetic interest which can be reviewed only briefly in this paper.

Most investigators agree that the procambial strands, destined to form the xylem and phloem of the veins, arise from the middle cell layer or layers of the lamina (cf. fig. 5). Although, as Smith (59) maintains, this may be the usual condition, several interesting exceptions deserve mention. Mounts (38) found that the veins in the leaf of *Vitis* and *Catalpa* arise from the uppermost or adaxial layer while Weidt (66) describes two distinct methods of origin of procambial strands in *Heterotrichum* (fig. 10). In general, if supporting tissue (collenchyma or fibers) accompanies the veins, it originates from the abaxial and adaxial subepidermal layers (12).

In view of the wide variation in the number, position and character of the cell layers in the mesophyll of angiosperm leaves, no uniform scheme of origin of this tissue region may be anticipated. However, reference to figs. 6, 8, 9, 10 will show that the palisade parenchyma, at least in the dorsiventral type of blade, usually arises directly from the adaxial subepidermal layer. According to Avery's (2) observations, cells of the palisade layer appear distinct in size "almost as soon as the lamina is initiated." In some cases the spongy parenchyma of the leaf may originate from the abaxial layer as well as from the non-vascular areas in the middle layers of cells (figs. 7-10). In other plants, such as *Bougainvillea*, the spongy parenchyma is formed by repeated periclinal division of cells derived from the original abaxial layer (fig. 6). As far as the writer is aware, no attention has been paid to the genesis of the mesophyll in the isolateral type of leaf, although information on this point would bear directly on various problems of "ecological anatomy."

In conclusion, brief mention should be made of certain aspects of tissue maturation which are of general physiological and anatomical interest. Recent studies have clearly shown, in the first place, that cell division and cell enlargement are unequal in duration and intensity in the various tissues of a maturing leaf. For example, the cells of the palisade parenchyma continue to divide for a longer period than either the spongy parenchyma or the epidermis (2, 38,

⁶ The upper and lower external layers are omitted from discussion since they invariably produce the epidermal system.

66). On the other hand, cell enlargement continues longer in the epidermis than in any other tissue (2, 38, 68). As a result of this inequality in growth of the various layers, it is assumed that "stresses" and "strains" arise which are responsible for the pulling apart of the subepidermal cells and the formation of the physiologically important air-spaces of the mesophyll. Mounts (38) concludes that "insufficient stress has heretofore been placed upon the mechanico-dynamic function of the epidermis" which appears to be "a major factor in leaf expansion." Avery (2) adopts a similar viewpoint, and in addition suggests that the wavy contour of the cells of the lower epidermis is produced by the reciprocal "pull" imposed upon them as the cells of the spongy parenchyma become separated. Further investigations, however, are urgently needed before we can view the complex process of tissue maturation entirely from such a "mechanical" viewpoint.

FOLIAR DETERMINATION IN ANGIOSPERMS

Although such diverse organs as bud scales, bracts and floral appendages have long been regarded as the homologues of "leaves," little detailed information is available regarding their mode of origin or cellular differentiation. The writer (9, 10, 11, 12, 13) has recently discussed this question and has applied the phrase "foliar determination" to the complex series of processes (cellular, genetic and physiological) which regulate the production of various foliar structures at the shoot apex. It must be admitted that we possess virtually no knowledge of the interaction of genetic and physiological factors during foliar differentiation. Although every cell at the growing point may be "omnipotential," the "prospective value" of the various cells and layers is doubtless limited by such factors as their relative position, nutrition, etc. (32, 49). An experimental attack on those problems is needed but great difficulty arises, with our present technique, because of the small size and delicate nature of the shoot growing point.

In spite of the difficulties inherent in the determination problem in plants, a survey of the comparative histogenesis of various types of foliar structures is first of all desirable. Several recent contributions to this aspect of the question deserve brief mention here. Schüepf (53) concludes, as a result of his experimental-developmental study on *Acer pseudoplatanus* L., that the "determination"

of bud scale or foliage leaf coincides with the emergence of the primordium at the growing point. He further indicates the importance of the type, position and duration of meristematic tissue in regulating the form and size of the adult organ. Schüepp's viewpoint has been adopted by Foster (12, 13) in his detailed investigation of bud scale and foliage leaf histogenesis in various species of *Carya*. In *C. Buckleyi* var. *arkansana* Sarg. a histogenetic divergence between scale and foliage leaf occurs when their respective primordia are less than .1 mm. in height. From this point, each foliar type embarks upon a specific and dissimilar career of differentiation. The "transition forms" likewise experience an early and specific differentiation characterized by the combination of histogenetic processes "normally confined, respectively, to the bud scale and foliage leaf."

Finally, attention must be drawn to the recent histogenetic studies on the flower by Grégoire (17, 20). He finds that in *Aquilegia*, *Ranunculus* and *Magnolia*, for example, the young receptacle consists of a massive parenchymatous core enveloped *both* apically and laterally by several layers of "germinative meristem," from which arise the primordia of petals, stamens and carpels. Thus, Grégoire argues, vegetative and floral "growing points" are fundamentally different in structure and the generally assumed homology between petals, stamens and carpels, and the foliage leaf does not exist.⁷ Although Grégoire's general viewpoint has been rejected by Troll (65), his observations are of interest and similar studies in a wide series of different floral types should be made. It seems only proper to conclude that a well-balanced interpretation of foliar structures in the angiosperms must consider and incorporate the evidence furnished by developmental studies (1, 10, 55).

EXPLANATORY NOTES

By the Editors

abaxial: the side of a lateral organ away from the axis.
acropetal: produced in a succession towards the apex.

⁷ Grégoire further develops this thesis in a more recent article, "Sporophylles et organes floraux, tige et axe floral" (Rec. Trav. Bot. Néerl. 32: 453-466, 1935), which has just come to the writer's attention. Particular emphasis is placed upon (a) the origin and early histogenesis of stamens and carpels and (b) the divergence in mode of vascularization and the "dishomology" between a leafy shoot and a flower. Grégoire rejects the classical interpretation that floral organs are sporophylls and concludes that stamens and carpels, respectively, are "microsporangiophores" and "spermatophores."

adaxial: the side of a lateral organ facing the axis.

anticlinal: perpendicular to the surface.

auxin *a*: one of the three growth-promoting chemical substances which have been isolated in crystalline form from plants. See Went's article in Bot. Rev. 1: 162-192. 1935.

basipetal: produced in a succession towards the base.

chimaera: the product from a bud involving mechanical coalescence of two different parental tissues.

histogenesis: the study of the origin and formation of tissues.

homologous: parts which are similar in origin regardless of their form or function.

periclinal: parallel to the surface.

periclinal chimaera: a graft-hybrid.

periblem: the cylinder of very young cells just inside the epidermis of a growing point, enclosing the plerome and developing into the cortex.

plerome: the central core of very young cells within the periblem at a growing point of roots and stems. It develops into the vascular and other tissues.

phyllotaxy (is): arrangement of leaves with respect to the axis.

procambial strands: strands of elongated cells which develop into food- and water-conducting cells.

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PLANTS MADE POISONOUS BY SELENIUM ABSORBED FROM THE SOIL

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It was rather alarming to read four years ago that grain from certain sections of the great cereal-growing region of the north central plains harbors the cause of a serious and sometimes fatal disease afflicting farm animals and possibly also man; that farm animals get the disease from eating hay and grain raised in the affected areas; and that flour and other mill products made from the grains might poison human beings (1). Equally startling was the statement that records of similar disorders have been found running back for many years, into early exploration days, but that until recently the malady was merely shoved aside as "alkali trouble" and not seriously considered (1). A review of the literature shows that a number of phases of this problem are being studied. Much is yet to be discovered, however, and many interesting problems remain to be solved by workers in botany, chemistry, veterinary science, public health, and government.

HISTORY

For many years a disease of livestock, including poultry, has been known west of the Missouri River in South Dakota and adjacent areas, and has been referred to as "alkali" disease, because of a relationship believed to exist between the alkali in the water or soil and the development of the disease. Apparently the earliest account of the malady is to be found in a report written in 1860 by Madison (39). Madison described the symptoms of a fatal disease which affected the cavalry horses, Indian ponies, and mules at Fort Randall, then in the Territory of Nebraska but now included in South Dakota. He attributed the disease to the pasturage. A recent study of the soils and plants of this area has shown that the conditions have not changed materially in the 80 years that have elapsed since Madison's observations (9).

The third annual report of the Wyoming Agricultural College and Experiment Station in 1893 alluded to a peculiar disease of horses in Carbon County, Wyoming (cf. 12). The symptoms were

typical of alkali disease, although at that time ascribed to ergot poisoning from hay.

Peters (44) in 1904 gave an accurate description of alkali disease in Boyd County, Nebraska, where it had been prevalent among all kinds of livestock since the settlement of that region in 1891. Corn from the affected farms was shipped to the Nebraska Experiment Station, where it was fed to hogs. In a short time it produced symptoms identical with those observed on the farms in Boyd County. Although Peters was apparently in error in concluding that only moldy corn was culpable, he reported testimony of farmers which incriminated sound corn, other grains, and pasturage.

Farmers in affected areas early learned to associate the disease with particular and often rather restricted tracts of land. Many of them believed that the disease was caused by something in the vegetation. A few continued to think that the toxic effects were due to alkali in the water which the animals received. But careful investigation in 1912 and 1913 served to eliminate the water as the cause (34, 35).

Lipp (37) in 1922 gave a brief but well written account of alkali disease in South Dakota west of the Missouri River. He stated that farmers living in the alkali areas were firmly convinced that the growing forage plants store a sufficient quantity of the alkalis to cause the disease. Dealers in grain and hay, he said, refused to purchase products raised in certain restricted areas. The only sure means of prevention consists in the use of feeds grown on areas where the disease is unknown.

Byers (9) gives an interesting historical account of the recent scientific study that led to the discovery of the cause of the disease. In 1928 Dr. K. W. Franke, of the South Dakota Experiment Station, began his important series of investigations which have definitely proved that alkali disease is produced by consumption of grain, forage, and fodder grown upon definite soil areas. Early in 1931 a cooperative study of the problem was begun, which involved work in the bureaus of Home Economics, Animal Industry, Plant Industry, and Chemistry and Soils. In May, 1931, at a meeting of the interbureau committee Dr. H. G. Knight suggested that selenium be looked for in the grain. Dr. W. O. Robinson was furnished a sample of toxic wheat. He analyzed this and found that

it contained from 10 to 12 p.p.m. (parts per million) of selenium (47). Selenium was also present in soils from the affected areas.

A preliminary field survey of the alkali disease of livestock was made in 1931 by Franke and others (27). The survey included the central and southwestern parts of South Dakota, parts of northern Nebraska, and the eastern edge of Wyoming. The toxicity of the grains and grasses was found to be correlated with soils derived from Pierre shale. The disease occurs over the entire area of these soils, but is not evenly distributed. It seems to be absent in some areas comprising many square miles. There also seem to be seasonal variations. Where the disease is severe, considerable livestock may die or have to be killed, and eggs do not hatch. In such localities the raising of hogs, horses, poultry, and even cattle has been discontinued, and tractor farming of small grain is the sole substitute. Grain known to have been produced on affected soil brings only minimum prices. Likewise, affected animals usually bring low prices when marketed. Owners of some badly affected farms have abandoned them, not being able to finance, lease, or sell them. In many cases, new renters, unfamiliar with the conditions, have had heavy losses within a year after moving to such farms.

In Wyoming the injury inflicted through poisonous vegetation is most serious on the ranges, and in some districts this injury has prevented profitable use of lands for grazing (4, 5, 6).

It is of historical interest that Crawford (11) in 1908 was probably the first person to suggest that certain obscure chronic diseases of livestock on our unirrigated plains might be caused by inorganic constituents of the forage plants. He called attention to another significant point—namely, that some “loco” plants, though usually toxic, were non-toxic when grown on certain soils. He believed that the inorganic constituents were responsible for the poisonous action of the “loco” plants *Astragalus mollissimus* and *Aragalus lamberti*, at least those collected at Hugo, Colo.—a region which Byers (9) has recently shown to be seleniferous. Crawford seems to have been incorrect, however, when he concluded that barium was the poisonous inorganic constituent of the plants.

CAUSE AND NATURE OF THE DISEASE

Alkali disease. In horses, cattle, and swine alkali disease manifests itself by a depressed rate of growth, a loss of hair (conspicu-

ous in the case of the mane and tail of horses), and an abnormal development of hoofs, followed in severe cases by the sloughing off of the old hoofs (27, 37, 44). The most severely alkali-d animals die or have to be destroyed. In poultry the malady results in non-hatchability of eggs. The few young that hatch are weak and have a greased appearance.

Selenium in wheat and soil. The first indication as to the nature of the toxic principle in cereals was published in 1933 by Robinson (47). He reported the occurrence of 10 to 12 p.p.m. of selenium in wheat and of 0.3 p.p.m. of selenium in soil from affected areas. Byers (8) found selenium, chromium, vanadium, and arsenic in soil from the same regions. In wheat from this locality he found selenium and vanadium.

Toxicity of plants containing selenium. Nelson, Hurd-Karrer, and Robinson (41) reported that plants absorb selenium from the soil, and become highly toxic to animals. Quantities of selenium as small as 1 p.p.m. in the soil, added as sodium selenate, permitted growth and maturation of wheat plants, with no visible symptoms of injury to the plants. However, when the grain or straw from these plants was fed to experimental animals, such as rats and guinea-pigs, it produced a pronounced toxicosis characterized by retardation in growth, and death occurred in a few weeks. Wheat which was found by analysis to contain 8 to 10 p.p.m. of selenium, absorbed from the soil, produced fatal injury with, in many cases, readily detectable macroscopic changes in the liver. Fifteen p.p.m. of selenium in the soil, added as sodium selenate, produced distinct chlorosis and stunting of wheat plants.

Toxicity of natural grains. Franke (16) has conducted extensive feeding experiments on white rats. He found that grain from farmers whose livestock had been affected was extremely toxic to white rats. The grain had no unusual odor or taste. The pathological symptoms produced in the animals were different from those brought about by any known plant toxin, such as alkaloid, glucoside, saponin, or toxalbumin. The toxicity could not be attributed to any product of decomposition in the grains, and certainly could not be ascribed to a deficiency of proteins, carbohydrates, fats, vitamins, or minerals.

Individual rats exhibited wide variations in degree of tolerance, and affected grain from different sources showed all degrees of

potency. Lethal grain produced very severe effects, with death occurring within eight days in some cases. Sublethal grain produced growth retardation and death only after a long period of time; the least toxic grain, however, brought about only growth retardation.

Tests with the most potent of the lethal grain produced death in 321 out of 325 rats. The grain included corn, wheat, barley, and emmer. Death occurred as follows:

16 per cent on or before	25th day
39 per cent on or before	40th day
71 per cent on or before	60th day
92 per cent on or before	100th day
25 per cent died between	14th and 34th days
36 per cent died between	34th and 54th days
16 per cent died between	54th and 74th days

Six lots of sublethal grain produced death in only six out of 52 rats by the 60th day.

Pathology. The pathology of rats that had been fed on poisonous grain is described in detail by Franke (16). Autopsies revealed conspicuous liver lesions. Less toxic samples of grain produced depression of growth as the only observable effect. Weaned rats placed on a diet containing a high percentage of lethal grain consumed only about one-fourth of the normal amount of food. Some of the rats lost weight rapidly; others gained weight slowly. Franke and Potter (24) proved that the pathological effect was not due merely to inanition. Rats fed on limited amounts of control grain showed no abnormalities other than stunted growth.

Varying concentration and time. Franke (18) made experiments on the effect of feeding toxic foodstuffs to white rats in varying amounts and for different time periods. It was found that a diet containing only 17.5 per cent of toxic grain produced definitely depressed growth rates and also caused a number of deaths. Concentrations of 35 per cent and more caused still greater depression of growth and a greater number of deaths. A study was made of the effect of diets containing toxic wheat for 30-, 20-, and 10-day periods, followed by control diet. Pathological symptoms resulted even from the 10-day period on the toxic diet, although normal growth was resumed when the rats were changed to the

control diet for 165 days. The damage to the internal organs was never repaired. The pathological changes decreased as the intake of toxic food decreased.

Alternation of toxic and non-toxic foodstuffs. In another set of experiments Franke (19) fed toxic and control foodstuffs alternately to rats. The intervals were 5-, 10-, and 15-day alternations of toxic and non-toxic diets. This alternate feeding gave growth and food consumption curves exhibiting rhythmic decreases and increases.

Selenium like natural toxicant. Franke and Potter (25) reported that the symptoms of selenium poisoning produced by feeding rats small quantities of sodium selenite in an otherwise normal diet are virtually identical with the symptoms produced by the natural plant toxicant. The evidence obtained in these experiments supports the idea that selenium is closely related to the natural toxicant. But, according to the authors, it has not yet been definitely proved that the toxicity of cereal grains is strictly proportional to their selenium content. The maximum selenium content of the naturally toxic grains from farms is about 30 p.p.m.; every grain sample known from feeding tests to be toxic gave a positive test for selenium, and non-toxic grain obtained from areas remotely separated from the affected areas gave negative tests (Franke and Painter, 22). It is possible that vanadium (Byers, 8) or molybdenum (Beath, Eppson, and Gilbert, 4) may contribute to the toxicity of some of the grains.

Effect of toxic grains on chickens. The low hatchability of eggs from chickens fed on toxic grains was studied by Franke and Tully (28). Monsters were produced from eggs obtained from hens on an affected farm. Approximately 75 per cent of the eggs which failed to hatch on the twenty-first day contained deformed embryos. The hatchability was only 4 to 12 per cent. Chicks from some of the eggs that hatched lived only a few hours. The down on these chicks appeared greasy and never became fluffy. When chicks were fed a ration containing 65 per cent of a toxic grain, distinctly inhibited growth resulted (Tully and Franke, 54). When they received only 25 per cent, they made practically normal growth. Egg production was delayed and reduced by the ration containing 65 per cent of affected grain. No distinct lesions of the internal organs were found from gross appearance as in the

case of rats. By injecting selenium into eggs before incubation, monsters have been produced, having beaks, eyes, and legs missing or malformed (Franke, 21).

Anemia. Anemia developed in white rats which were fed toxic wheat (Franke and Potter, 24, 25). The decline in hemoglobin was shown not to be due to inanition.

Avoidance of toxic food. Rats on a toxic diet voluntarily reduced their food intake (16, 24, 25). In experiments in which rats were given a choice between diets having various concentrations of selenium (natural or sodium selenite), the animals invariably chose the least toxic food, possibly basing their selection on taste or odor imperceptible to man (26). It is commonly believed by cattlemen that range animals are able to recognize the seleniferous vegetation (some of which possesses a telltale garlicky odor), and eat only the least toxic.

Selenium poisoning and oxidation-reduction relations. Schneider (48) investigated the toxic effects on rats of ingested sodium selenite incorporated in a stock diet. At a level of 70 p.p.m. it arrested the growth of 3-weeks-old albino rats and caused emaciation and death within 2 weeks. A level of 35 p.p.m. caused slight and erratic growth, death occurring in about 6 weeks. A level of 17.5 p.p.m. resulted in slightly subnormal growth and a variation in the lethal effect; some of these animals lived as long as 267 days, with no other symptoms than a slightly subnormal growth; these growth effects were shown to be due to inanition.

Adult rats withstood the poison more effectively than growing rats. On high levels, of 420 p.p.m., adult rats virtually starved to death, although autopsy revealed subacute pathological changes. The symptoms of selenium poisoning described by Schneider are apparently identical with those reported by Franke (16) for the naturally toxic grain. Schneider suggests that selenium may disturb the oxidation-reduction relations of physiology, in which sulphur normally plays an important part. He was able to show a diminished oxygen absorption by rat liver to which sodium selenite was added, but could demonstrate no diminution in the livers of rats which had previously received a selenium ration.

Further evidence that selenium is capable of inhibiting cellular respiration is seen in the work of Potter and Elvehjem (45) which showed that the rate of oxygen uptake by yeast was reduced to

one-fifth by the addition of sodium selenite when glucose, mannose, or fructose was used as substrate. The inhibition was most marked in an acid medium and fell off rapidly at pH 7.5.

Selenium in Astragalus. Beath, Draize, and Eppson (3) pointed out in 1932 that specimens of *Astragalus bisulcatus* (the two-grooved milk vetch) growing in some soils had an extremely offensive garlicky odor, while specimens from other soils entirely lacked this odor. It was later demonstrated (5) that the plants with the offensive odor were more toxic than those lacking it. The only variable factor contributing to this difference was the presence of selenium. This plant seems to be responsible for more losses of cattle and sheep in Wyoming than are caused by any other poisonous plant.

Indicator plants. Eight species of native plants, including *Astragalus bisulcatus*, were reported as indicator plants that always contain selenium when collected on soils derived from Cretaceous and Eocene shales (5). The indicator plants were richest in selenium when they occurred on the undecomposed shales. The selenium content of the plants varied from a trace to 1000 p.p.m. Range plants containing selenium were more poisonous to livestock when they grew on the Niobrara, Steele, or Pierre shales than when they grew on the other shale formations.

Since it contains an alkaloidal poison, *Astragalus bisulcatus* free from selenium is somewhat poisonous to livestock. Forced feeding trials demonstrated that 50 ounces of selenium-free *A. bisulcatus*, per hundredweight of sheep, failed to affect an animal seriously, whereas 25 ounces of the same species containing selenium produced death in a few hours.

Selenium poisoning. There appears to be some variation in the selenium poisoning of livestock, depending upon the species of plant ingested (5). This suggests that the various selenium-bearing plants carry the element in different chemical combinations, or that the selenium may be present with other toxic substances. But, in general, the autopsy findings of animals dying on the range of blind staggers (the term used in Wyoming to denote this type of poisoning) are in fairly good agreement with those of the experimental animals dying from the administration of small quantities of the sodium salt of selenious acid.

Types of disease. Draize and Beath (12) distinguish two types

of disease in livestock due to mineral poisoning—namely, alkali disease and blind staggers. Alkali disease, well known in South Dakota but rather uncommon in Wyoming, is the less acute form and is characterized by loss of hair and deformation and sloughing off of hoofs. Blind staggers, a disease of Wyoming range cattle, represents a much more acute type of poisoning, which results in death within a comparatively short period of time. There is no sloughing of hoofs or loss of hair in typical cases of blind staggers. Both diseases, however, are characterized by similar injuries to the liver. These authors believe that the two forms of disease are produced by the same agent, and that microscopic pathology hardly justifies their designation as distinct types of poisoning.

Selenium in animal body. Selenium, when present in food, finds its way into all the body tissues, attaining concentrations as high as 16 p.p.m. in the heart, 25 p.p.m. in the liver, and 27 p.p.m. in the blood (Dudley and Byers, 15; Byers, 9). Since selenium is present in all secretions and excretions, selenium poisoning does not seem to be permanently cumulative, even though injury to the tissues is permanent in character. According to Dudley (13), the blood of animals fed inorganic or organic compounds of selenium may contain from 7 to 27 p.p.m., thus transporting the toxic material to all parts of the body. The selenium, however, is deposited predominantly in the liver, kidney, and spleen (3 to 25 p.p.m.). Concentrations of 8 to 20 p.p.m. were also found in the hoofs. Since the bile and urine may contain as high as 5 or 6 p.p.m., excretion by hepatic and renal routes seems mainly responsible for elimination of selenium from the body. Dudley (14) found that the urine of men employed in the extraction of selenium contained from a trace to 6.9 p.p.m. A garlicky odor of the breath and other symptoms were noted.

Fermentation. Franke and Moxon (20) studied enzyme activity in order to develop a simple biological test for the toxicity of proteins, and to study possible effects of these toxic proteins on several important enzymes of the animal body. They found that protein from normal grain, when added to a fermenting mixture of yeast and glucose, would increase the rate of fermentation, whereas protein from an affected grain would not increase the rate of fermentation. Moxon and Franke (40), in reporting studies on the effect of selenium salts on fermentation, state that the toxicity of

sodium salts of selenite, selenide, and selenate decreases in the order named.

Water. Alkali water has been definitely eliminated as a possible cause of either the so-called alkali disease or blind staggers (4, 30, 34, 35). The water itself contains no appreciable amount of selenium or other toxic substance, although water with a high salt content may possibly aggravate the effect of toxic foodstuffs. In Wyoming the popular belief that the water is poisonous probably arises from the fact that selenium-bearing range plants frequently occur rather abundantly on the slopes of alkali basins (4).

Locoism distinct. Locoism, in Wyoming at least, does not seem to be due to selenium (4). The principal loco weeds in Wyoming are *Oxytropis saximontana* (white loco) and *O. bilocularis* (purple loco). These plants usually grow on soils derived from granites, sandstones, and volcanic ash. They sometimes grow on Cretaceous shales, and then give a positive test for selenium. Loco weeds free from selenium, when consumed in large quantities, produce the loco disease after about 50 days. The symptoms are different, however, from those of blind staggers.

DISTRIBUTION OF SELENIUM IN SOILS

Areas involved. Byers (9) has shown that soil areas of enormous extent contain selenium. It has already been demonstrated in soils and vegetation in South Dakota, Nebraska, Wyoming, Montana, Colorado, Kansas, Oklahoma, Utah, Arizona, and New Mexico. Further investigation will probably show a wide extension of the affected areas in many parts of the world. The distribution of selenium in nature is discussed by Strock (53).

Source of selenium. The source of selenium in soils has been shown by Byers (9) to be pyrite or similar minerals occurring in the geological formations that produce the soil. The seleniferous soils are derived, for the most part, from shales of the Cretaceous period. Such soils appear to retain enough selenium to produce toxic vegetation when the mean annual rainfall is insufficient to produce percolation through the soil profile. Seleniferous soils may be expected in arid and semiarid regions where the soils are derived from selenium-bearing strata. They probably will be found in a number of the great wheat-producing areas of the world. There are definite indications that seleniferous soils actually exist in four continents, at least.

The distribution of the selenium is not uniform, either in the surface soil or in the soil profile. There are indications of a zone of selenium concentration in the soil profile, either within or close to a zone of sulphate accumulation. Sandy soils will probably be found to contain selenium in only small quantities, because of low original content and more complete leaching than from heavy clay soils.

Geology. Beath *et al.* (4) have shown that selenium is distributed in Wyoming throughout shales of the Cretaceous and Eocene periods, and that its concentration is comparatively high in Steele, Pierre, and Niobrara formations. These writers give an excellent description of the geology of Wyoming shales, based on the work of Dr. S. H. Knight, state geologist of Wyoming. Beath (2) has also found selenium in red soil derived from Chugwater, which is of the Permian or Triassic (?) age.

Soil enrichment. Although Niobrara shale *in situ* usually carries only 2 to 4 p.p.m. of selenium, soils derived from this shale may show an enrichment which results apparently from drainage of soluble residues of selenium-bearing plants (4). Soil at a depth of 36 inches contained 22.5 p.p.m. of selenium, of which approximately one-third was water-soluble.

Fertilizers and insecticides. Selenium may occur in soils to which it has been added as an impurity in superphosphate and ammonium sulphate (46, 49, 50, 51) and in soils contaminated with fungicides and insecticides containing it (29, 38, 41, 52).

ABSORPTION AND ACCUMULATION OF SELENIUM BY PLANTS

Influential conditions. The absorption and accumulation of selenium by plants growing on seleniferous soils are dependent upon two groups of conditions. I. Soil conditions: (1) Concentration of selenium in the soil; (2) nature of the selenium compounds, especially their solubility in water; (3) other soil components, especially available sulphur; and (4) seasonal variation in rainfall. II. Plant conditions: (1) Kind of plant; (2) part of the plant examined; and (3) stage of growth.

Plant symptoms. Studies by Hurd-Karrer (32) showed that the characteristic symptom of selenium injury in wheat plants is a snow-white chlorosis. This was produced by sublethal concentrations of sodium selenate (15 to about 30 p.p.m. selenium in Key-

port clay loam). When the selenate was added to pots containing older plants, the white chlorosis appeared only on leaves emerging subsequent to the addition of selenium, those already formed merely turning yellow. A progressive diminution of chlorosis on successive leaves was observed as the plants became older.

Absence of symptoms in the field. It is worthy of emphasis in this connection that symptoms of selenium injury of plants have never been reported from observations in the field. One would never suspect from the appearance of wheat, cabbages, etc., that they have accumulated dangerous quantities of selenium.

Replacement of sulphur. Cameron (10) suggested that selenium might produce its injury to plants by replacing sulphur in some organic compound. Levine (36) also considered that selenium might replace sulphur in plant metabolism. Brenner (7) thought that it might replace sulphur in the metabolism of sulphur bacteria.

Selenium in protein. Selenium absorbed by wheat is associated with the protein of the grain, possibly replacing sulphur (Nelson, Hurd-Karrer, and Robinson, 41). Franke (17) showed that the toxicant is carried in the protein fraction of toxic wheat and toxic corn. This was determined by feeding experiments. Franke and Painter (22) found selenium in the proteins of toxic corn, wheat, and barley, and in the proteins of animals that had been fed toxic foodstuffs. The selenium was in organic combination in the protein (probably replacing sulphur) and was in solution after the protein had been hydrolyzed. Painter and Franke (43) developed a method of removing selenium from toxic protein hydrolysates, through the use of butyl-alcohol extraction. A procedure with mercuric chloride was devised whereby all the selenium compounds were precipitated, mercury salts being superior to other amino-acid precipitants. The molar selenium-sulphur ratio, according to Painter and Franke (42), in a toxic protein investigated was 1:148. The effect of chemical treatment of the proteins from toxic wheat was studied by animal feeding (Franke and Painter, 23). The hydrolysates of toxic proteins were toxic to rats. But mercuric chloride precipitation of toxic hydrolysates removed the selenium sufficiently to render the filtrate innocuous when fed to rats.

Sulphur antagonism. Hurd-Karrer (31, 32) made an important discovery when she found that the toxicity of sodium selenate

is determined by the amount of sulphur available to the plants. In water cultures, selenium concentrations as low as 0.1 p.p.m. produced distinct injury in wheat plants after a few weeks with nutrient solutions containing no sulphate, whereas a concentration of 18 p.p.m. was required for this degree of injury in solutions containing 192 p.p.m. sulphur. There was no visible injury to the plants where the proportion of selenium to sulphur was 1:12 or less, the point of minimum detectable injury lying between 1:9 and 1:11. When the ratio was 1:8 or greater, the plants were chlorotic and stunted; and when the ratio was as high as 1:2, growth was almost completely inhibited. In soil cultures, selenium injury could be inhibited by the addition of excess sulphur, as sulphates or as elemental sulphur. The amount required varied with the toxicity of the selenate in the particular soil.

Chemical analyses showed that the injured plants contained large amounts of selenium in their tissues, whereas plants grown with these same concentrations of selenium, but with sufficient sulphates or sulphur to reduce or prevent the injury, contained much less (32). The absorption of selenium appeared to be determined not by its absolute concentration in the soil or nutrient solution but by its relative concentration with reference to the available sulphur. Applications of gypsum or elemental sulphur reduced the absorption by wheat of the naturally occurring selenium in the soil, as well as of that added as sodium selenate (33). This selenium-sulphur relationship seems to constitute a hitherto unreported instance of antagonism.

It has not been proved, however, by field tests that application of sulphur to seleniferous cultivated land offers a practical means of preventing grains from accumulating toxic concentrations of selenium.

Conditions governing absorption. Further greenhouse experiments by Hurd-Karrer (33) illustrated the effects of various conditions on the absorption and resulting toxicity to wheat of selenium added as sodium selenate to the soil. These included soil type, percentage of sand, method of adding selenium, the form of selenium added, and growth of previous crops. Sodium selenate was more easily absorbed by wheat from Pierre clay (a naturally seleniferous soil) than from Keyport clay loam. The greatest accumulation was 1350 p.p.m., found in plants fatally injured by

20 p.p.m. of selenium as sodium selenate added to Pierre clay. The addition of quartz sand to Keyport clay loam increased the toxicity of sodium selenate in proportion to the percentage of sand in the mixture. Sodium selenate was not easily leached from Keyport clay loam, being at least partially retained in the upper layers when a solution containing it was poured on the surface; solutions originally toxic were found to be non-toxic after being filtered through this soil. The presence of sand increased penetrability. Elemental selenium was apparently unavailable and non-toxic to wheat plants, at least in quantities up to 200 p.p.m. in Keyport clay loam. The selenium in sodium selenate proved to be more available and more toxic to wheat than that in sodium selenite. The growth of successive crops of wheat changed sodium selenate to a less toxic form or reduced it to a subtoxic concentration.

Field surveys. The extensive field survey of Byers (9) showed that wherever selenium was present in the soil, all kinds of plants absorbed some of this element, in amounts varying from mere traces to thousands of parts per million. Sufficient data are not yet available for an exact statement, but in general it appears that any soil containing more than 0.5 p.p.m. of selenium or any vegetation containing over 5 p.p.m. is potentially dangerous. The possibility of injury to the consumer depends of course upon the fraction of seleniferous food in the total diet.

All soils that contain selenium do not produce toxic plant materials, according to Byers (9). Certain areas bear little or no toxic vegetation even where considerable quantities of selenium occur in the soil. These unexplained variations suggest an important problem for further research. In some cases a high sulphur-selenium ratio of the soil seemed clearly to limit the accumulation of selenium in the plants.

Differences in the ability of plants to accumulate selenium. Analyses presented by Byers (9) demonstrate striking differences in the ability of various plants to accumulate selenium. Certain plant species, such as native prairie grasses, show relatively slight ability to take up selenium. Other species, including some legumes and composites, show a marked tendency toward ready absorption. The cultivated cereals seem to be intermediate in character. Some examples may be cited: Western wheat grass growing on various soils accumulated from 1 to 60 p.p.m. of selenium. *Astragalus*

bisulcatus (a wild legume) accumulated from 200 to 4300 p.p.m. A striking contrast is obtained when we compare two closely related species of *Astragalus*. Thus *Astragalus bisulcatus* on soil containing 2.1 p.p.m. stored 1250 p.p.m., while *A. missouriensis* on the same soil accumulated only 3.1 p.p.m.

A conspicuous feature of the data is the general indefiniteness of correlation between the selenium content of the soil and the selenium content of a given kind of plant (9). For example, from one soil containing 2.5 p.p.m. of selenium wheat accumulated 45 p.p.m.; from another soil containing 3.0 p.p.m. wheat took up only 0.5 p.p.m. From six soils with 1.5 p.p.m., wheat absorbed 1, 1, 2, 7, 7, and 10 p.p.m. From a soil containing 6 p.p.m. Western wheat grass absorbed 60 p.p.m., whereas from a soil with 20 p.p.m. this plant took up only 12 p.p.m. For Western wheat grass the ratio of selenium in the plant to that in the soil in twenty-two cases varied from 0.3 to 14.0, with no indication of a mean. Chemical analysis of the soil would therefore not enable one to predict the amount of selenium that wheat, Western wheat grass, alfalfa, *Astragalus bisulcatus*, etc., would absorb. This low degree of correlation may result from differences in availability of the selenium, in the effects of sulphur or other soil constituents, in distribution not represented by the sampling, in age of the plants, or in other unknown conditions. It suggests very interesting problems for further research.

Beath, Eppson, and Gilbert (4) divide species of native plants in Wyoming into three groups, according to their ability to accumulate selenium: (1) Species of the first group invariably contain selenium when collected on Cretaceous and Eocene shales. This group includes *Astragalus bisulcatus* and five other species of *Astragalus*, *Mentzelia decapetala*, *Oonopsis condensata*, two species of *Xylorhiza*, and two species of *Stanleya*. The highest recorded content is for a sample of *Astragalus bisulcatus* with 8840 p.p.m. (2) Species of the second group of native plants vary in their selenium absorption, with tests in some cases negative and in others positive. These plants include five species of *Atriplex*, native grasses of various kinds, and twelve other species. (3) Species of the third group, which include thus far 33 kinds of native plants, contain no selenium even when growing on seleniferous soils.

It is remarkable that four species of *Astragalus* collected on the

same shales and in close proximity to the selenium-bearing species have consistently been found to be free from selenium or to contain mere traces (4). No clue has yet been found to these striking differences in the ability of closely related species to absorb and accumulate selenium. This constitutes one of the most interesting of the unsolved problems.

Hurd-Karrer (33) grew seventeen different crop plants in Keyport clay loam with 5 p.p.m. selenium added as sodium selenate. Broccoli and mustard, of the Cruciferae, absorbed more selenium than any others—i.e., 1180 and 1240 p.p.m., respectively. She suggested that the tendency of a crop to absorb selenium depends on its tendency to absorb sulphur.

Selenium converters. Beath and his associates (4, 5, 6) have advanced a very interesting hypothesis regarding the part played by certain native plants. Their idea is that certain native selenium-bearing plants, such as *Astragalus bisulcatus*, absorb selenium from virgin shale soils, convert it into water-soluble forms, and return it to the soil in forms available for absorption by other types of plants, including farm crops. These native plants are therefore regarded as selenium converters and soil contaminators.

The toxicants of the selenium-bearing range plants could be extracted freely with water. Through the decay of foliage, seeds, and roots of these plants a considerable amount of this element may be supposed to go back to the soil in forms readily available to any type of plant. The chemical nature of the water-soluble selenium compounds has not been described thus far, nor is it known what substances are responsible for the offensive odor of *Astragalus bisulcatus* and *Oonopsis condensata*. Water extracts of green *A. bisulcatus* mixed with crude undecomposed Niobrara shale in experimental plots imparted recognizable amounts of selenium to barley grown on the plots. Barley grown on the same shale composite without the addition of the vetch extract did not absorb selenium. Native grasses growing in close proximity to selenium-bearing range plants were found to be poisonous to guinea pigs. A number of other native plants were found to contain selenium when influenced by the plants that yielded soluble selenium compounds, but the same species of plants were selenium-free when grown on uncontaminated shales.

Selenium absorption by crop plants. The absorption of appre-

ciable amounts of selenium by farm crops, such as cereals and forages, is regarded by Beath and his co-workers (4) as dependent upon previous enrichment of the soil by converter plants. Tests showed that wheat and oats absorbed dangerous quantities of selenium if sown on soil prepared by ploughing under a heavy stand of native selenium-bearing plants. Whenever toxic corn, oats, wheat, and cultivated forage crops have been found in Wyoming, it has been possible to obtain evidence that the virgin soil recently supported dense growth of selenium converters, or had been enriched by drainage from these plants (4). Ordinary crops absorb little or no selenium in most of the farming areas of Wyoming, even though the soils themselves show the presence of selenium. This indicates that selenium is rarely, if ever, present in these areas in a form available to ordinary cultivated plants.

Small absorption from raw shales. Farm crops were grown upon raw Cretaceous shales taken from naturally occurring deposits so selected as to exclude any possibility of their having previously carried a growth of vegetation (4). It was found that these plants contained only very small quantities of selenium. The highest concentration was 2 p.p.m., in wheat heads. Some other crop plants, such as alfalfa, cabbage, sugar beets, and hairy vetch, contained no selenium. Peas contained 1.7 p.p.m.; crested wheat grass, 0.3; oat heads, 0.7; corn, 0.6; beans, 0.4; carrots, 0.3; parsnips, 0.2; rutabagas, 0.5; barley heads, 0.1; potatoes, 0.1; and sunflower seeds, 0.6.

It seems unlikely to Beath and his associates (4) that cultivated crop plants are rendered poisonous to livestock by the small quantities of available selenium present in soils derived from raw shales. Unfortunately, precise information on what constitutes a harmful amount of selenium in such crops is not yet available. It may be said, however, that barley hay containing 6 p.p.m. of selenium was fed to cattle for several months without producing symptoms of injury, and that salt bush containing 19 p.p.m. of selenium was fed to cattle and hogs for three months without noticeable ill effects. Attempts to produce alkali disease in cattle, sheep, and hogs by feeding wheat and barley containing only a few parts per million of selenium were unsuccessful.

Although Byers (8) reported that wheat accumulated 25 p.p.m. of selenium when grown on Pierre loam containing 2 p.p.m., Beath

and his associates (4) found that wheat accumulated only 2 p.p.m. of selenium when growing on raw Steele shale containing 2.4 p.p.m. To enable wheat plants to accumulate 25 p.p.m. of selenium, raw shale had to be sufficiently enriched with concretions to raise the selenium content to 65 p.p.m.

Action of soil contaminators. A striking contrast to the preceding results is seen in an experiment in which wheat was grown on a shale soil that had previously borne a dense growth of the selenium converters *Astragalus bisulcatus* and *A. pectinatus* (4). Although the soil contained only 1.1 p.p.m. of selenium, this appeared to be in a form easily available to wheat, for the plants accumulated 45 p.p.m. In another experiment a crop of barley was grown on a soil that had carried a heavy stand of *Astragalus*. The straw and grain, when fed to cattle, produced typical symptoms of alkali disease after six weeks' time. Chickens that ate the grain lost their feathers and many of them died.

Alfalfa. Alfalfa has not been reported as producing selenium poisoning of livestock in Wyoming (4). Chemical analyses of alfalfa from selenium-bearing raw shales have been negative or have shown only a few parts per million of selenium. Even when alfalfa was grown on a plot that previously bore a dense stand of *Astragalus pectinatus* and *A. bisulcatus*, the crop gave negative tests for selenium, both by chemical analyses and feeding trials with rabbits for two months.

Variations. Although, in general, the abundant forage plants on the ranges in Wyoming are free from dangerous quantities of selenium, there are some interesting and as yet unexplained variations. For example, *Eurotia lanata*, one of the most desirable forages on Wyoming ranges, was found to contain 23 p.p.m. of selenium on one Niobrara outcrop, whereas it was entirely free from selenium on a similar soil. Certain rather limited grazing areas in Wyoming are so dangerous that they have come to be known as poison areas. They usually bear dense growths of woody aster, *Oenopsis*, and species of *Astragalus*, and there are indications that even some of the native grasses may contain sufficient selenium to be injurious.

Is the toxicity of grains always dependent upon action of selenium converters? Although Beath and associates (4) have presented abundant evidence that converter plants are able to make

selenium available to crops, it has not yet been proved that the toxicity of grains in seleniferous areas outside of Wyoming is dependent upon the action of converter plants. Some of the soils that now produce toxic grain have been in cultivation for the last twenty-five or fifty years, and certainly have not recently supported dense stands of *Astragalus bisulcatus* or other selenium-accumulating wild plants. Further research will be needed to show how important a rôle the converter plants may have played in the cereal-growing regions where alkali disease has been prevalent for so many years.

CONTROL AND PREVENTION

Much more research work will need to be done before adequate methods of control and prevention of selenium poisoning can be applied. The following control measures have been suggested by Franke *et al.* (27): Toxic grain, hay, or grass should not be used for feed or pasturage. Affected animals should be transferred to unaffected areas. Areas definitely known to produce toxic grain and forage should be left uncultivated, and suspected areas should be studied to determine definitely whether or not they produce toxic vegetation.

Byers (9) has added the following recommendations: It would seem wise to withdraw toxic arable land from cereal production. The exact extent of affected areas should be determined, and remedial measures applicable to field conditions should be sought. Irrigation areas should be examined and controlled. Stock raising in seleniferous areas seems practicable if the ranges are not overgrazed. Analyses have shown that as a rule the native forage crops, such as buffalo grass, grama grass, bluestem, and needlegrass, contain relatively small quantities of selenium. Observations have indicated that animals learn to avoid seleniferous vegetation; even pigs avoid foods containing small quantities of inorganic selenium compounds (cf. 26). A study of toxic limits, tolerance limits, diagnostic symptoms, and remedial measures should be undertaken. Tolerance limits should be set up as soon as possible; but the emergency is not serious enough to warrant hasty measures. Above all, the proper safe-guarding of public health within the affected areas should be studied.

Beath *et al.* (4) suggest the following corrective measures as

applicable to Wyoming: Grazing of livestock should be avoided on areas bearing dense stands of seleniferous native range plants, and over-grazing should be prevented. Destruction of dangerous plants on desirable ranges by grubbing may be practicable in some areas; these plants are poisonous themselves, and they convert selenium into water-soluble forms that may be absorbed by desirable range plants. Revegetating with forages which are not selenium accumulators may be feasible. Ranchers should be advised regarding the kind of livestock best adapted to particular ranges. Cultivated fields that produce crops and forages toxic to livestock should be left uncropped until more information is available concerning types of feed and forage plants suitable for use on such areas, and until more is known about the amount of seleniferous grain and hay which the animals may tolerate. Although addition of sulphur to cultivated land offers a possible means of preventing grains and forages from absorbing toxic quantities of selenium, the shales and the soils derived from them are normally sulphurized, and successful treatment by this method may not be practicable. In the large grazing areas soil detoxification obviously would be too expensive. Drainage may serve to reduce the selenium content of irrigated soils.

POSSIBILITY OF HUMAN INJURY

Selenium in foods. Byers (9) gives a table that shows potentially dangerous selenium concentrations in some common foods from a naturally seleniferous area. The highest selenium contents, in p.p.m., are as follows: Cabbage, 100; turnip leaves, 25; eggs, 10; lettuce, 7; wheat, 5; rye, 5; corn, 3; string beans, 2; and milk, 1.5. Franke and Painter (23) report 31 p.p.m. in whole wheat and 120 p.p.m. in the protein of the grain. Using soil fortified by the addition of 5 p.p.m. of selenium, as sodium selenate, Hurd-Karrer (33) obtained much higher values, which are as follows, in p.p.m.: Mustard, 1240; broccoli, 1180; spinach, 315; young wheat, 470; young barley, 450; and young corn, 275.

Affected areas. In the more seriously affected areas human injury might be extensive if the inhabitants provided a considerable fraction of their diet from their own produce (9). Small mills, however, no longer exist, and the farmer receives his flour from Minneapolis or other distant milling centers. Relatively few vegetables are raised locally.

Country as a whole. The danger to public health outside the selenium area seems relatively slight. Even when toxic wheat is marketed, its dilution with non-toxic wheat and the small fraction which bread constitutes of the normal diet would tend to render it practically innocuous (9). The problem deserves careful study. Methods need to be devised for diagnosis of incipient selenium poisoning (Dudley and Byers, 15). Tolerance limits, toxic limits, and remedial measures should receive thorough investigation. The research required on human phases of the problem seems barely to have been touched. The problem is not confined to the United States; for, according to Wilcox (55), selenium has already been found in wheat from Canada, Mexico, Argentina, Australia, New Zealand, South Africa, Algeria, Morocco, Spain, and Bulgaria.

Insecticides. Nelson, Hurd-Karrer, and Robinson (41) have warned against the use of selenium compounds as insecticides, since there is considerable danger from even minute quantities of selenium in soils on which food products are grown. Even spray residues ordinarily considered innocuous may be made available to the plant and be accumulated in toxic amounts.

TOXICITY OF OTHER MINERAL ELEMENTS

Molybdenum. Most plants growing on Cretaceous shales have been found by Beath, Eppson, and Gilbert (4) to absorb molybdenum in varying amounts. Some plant species, particularly *Oenopsis condensata* and *Xylorhiza parrii*, contain fairly large quantities. One sample of *Oenopsis* showed 317 p.p.m. of molybdenum. When barley was grown on soil to which sodium molybdate had been added, the hay contained 89 p.p.m. of molybdenum. The hay was fed to livestock, and produced erosion of long bones and some other symptoms similar to those brought about by selenium. Morrison shale, even when uncontaminated through the activity of *Astragalus bisulcatus*, etc., seems to produce poisonous cereals, root crops, and forages. The toxicity of these crop plants seems not to be dependent on their selenium content. Possibly molybdenum is responsible.

Tellurium. Tellurium is found in only a few native plants in Wyoming (4). A cactus showed 25 p.p.m. and salt bush 2 p.p.m. No tellurium has been found in crop plants growing on farms, but wheat seedlings grown in soil containing potassium tellurite accu-

mulated 507 p.p.m. It is to be expected that plants containing tellurium will be much less toxic than those containing selenium.

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